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Morphological and Phylogenetic Characterizations of Freshwater *Thioploca* Species from Lake Biwa, Japan, and Lake Constance, Germany

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Filamentous, gliding, sulfide-oxidizing bacteria of the genus *Thioploca* were found on sediments in profundal areas of Lake Biwa, a Japanese freshwater mesotrophic lake, and were characterized morphologically and phylogenetically. The Lake Biwa *Thioploca* resembled morphologically *Thioploca ingrica*, a brackish water species from a Danish fjord. The diameters of individual trichomes were 3 to 5.6 μm; the diameters of complete *Thioploca* filaments ranged from 18 to 75 μm. The cell lengths ranged from 1.2 to 3.8 μm. In transmission electron microscope specimens stained with uranyl acetate, dense intracellular particles were found, which did not show any positive signals for phosphorus and sulfur in an X-ray analysis. The 16S rRNA gene of the *Thioploca* from Lake Biwa was amplified by using newly designed *Thioploca*-specific primers (706-Thioploca, Biwa160F, and Biwa829R) in combination with general bacterial primers in order to avoid nonspecific amplification of contaminating bacterial DNA. Denaturing gradient gel electrophoresis (DGGE) analysis of the three overlapping PCR products resulted in single DGGE bands, indicating that a single 16S rRNA gene had been amplified. With the same method, the *Thioploca* from Lake Constance was examined. The 16S rRNA sequence was verified by performing fluorescence in situ hybridization targeted at specific motifs of the Lake Biwa *Thioploca*. Positive signals were obtained with the bacterial probe EUB-338, the γ-proteobacterial probe GAM42a, and probe Biwa829 targeting the Lake Biwa *Thioploca*. Based on the nearly complete 16S rRNA sequence and on morphological similarities, the *Thioploca* from Lake Biwa and the *Thioploca* from Lake Constance are closely related to *T. ingrica* and to each other.

Members of the genus *Thioploca* are gliding, filamentous, sulfur-oxidizing bacteria that are distinguished from the related genus *Beggiatoa* by a morphological trait, the common sheath that surrounds the trichomes (11, 17). Since the discovery of dense mats of large *Thioploca* spp. on the continental shelf of South America (7), the extraordinarily dense and widespread communities of these bacteria have been surveyed and described in more detail (5, 10, 20, 27, 29, 31, 32, 34). Most of the current information about this genus, including phylogenetic relationships and ecological and physiological traits, has been gained from studies of marine *Thioploca* mats in coastal upwelling areas, the largest recognized habitat.

Although pure cultures have not been obtained so far, physiological features of marine *Thioploca* spp. have been revealed to some extent by experiments performed with freshly collected *Thioploca* samples (20, 27). *Thioploca* cells accumulate high concentrations of nitrate in large intracytoplasmatic vacuoles and reduce the nitrate to ammonium, with concurrent oxidization of sulfide to sulfate (27). *Thioploca* is capable of autotrophic carbon fixation, but it can also grow mixotrophically and assimilates substrates such as acetate and amino acids (20). Considering their large biomass and their physiological capabilities, *Thioploca* mats play a critical role as a link for sulfur, nitrogen, and carbon cycles in marine coastal ecosystems.

On the other hand, the phylogenic relationships and the physiology of freshwater *Thioploca* are still unknown, although the genus originally was described from freshwater lakes and rivers (12, 13, 15, 37). Many of the previous *Thioploca* freshwater communities have declined or disappeared, in part due to human impact, and are no longer available for study (18). However, mats of *Thioploca* spp. were recently found in Lake Biwa, a Japanese freshwater lake (26). This finding provides an opportunity to investigate freshwater *Thioploca* in detail. In this study, a *Thioploca* sp. from Lake Biwa was characterized morphologically and by 16S rRNA sequencing in order to provide the basis for further detailed studies. In addition, a *Thioploca* sp. from Lake Constance, Germany, was characterized by 16S rRNA sequencing in order to analyze phylogenetic relationships among freshwater *Thioploca* spp. from different geographical locations.

MATERIALS AND METHODS

Sampling sites and collection of *Thioploca* specimens. *Thioploca* spp. were collected at station A (35°23.4′N, 136° 7.7′E) at a depth of 90 m in the northern part of Lake Biwa. Sediment samples were obtained with a Ekman-Birge grab sampler (15 by 15 cm). *Thioploca* filaments (sheaths with trichomes) were also collected with a dredge net originally used for collection of macrozoobenthos (26). After 5 min of dredging, numerous *Thioploca* filaments accumulated on the front part of the dredge net. Dense clumps of filaments on the frame of the dredge net were collected with forceps and immediately transferred into a container with sediment and water from the collection site.

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The sediment sample from Lake Constance that contained *Thioploca* filaments was collected by a diver at a depth of 22 m near the Island of Mainau (Germany).

Light microscopy. The morphological traits of *Thioploca* were observed by light microscopy. Micrographs were taken with a phase-contrast microscope (Axioplan; Zeiss, Göttingen, Germany) equipped with a charge-coupled device digital camera (Axiocam; Zeiss).

TEM and X-ray analysis. *Thioploca* bundles from Lake Biwa used for transmission electron microscopy (TEM) observation were fixed, dehydrated, and embedded as described previously (19). Thin sections were cut with a diamond knife, mounted on 100- or 200-mesh copper grids, and poststained with uranyl acetate and lead citrate. TEM micrographs were taken with a JEM 1010 microscope (JEOL, Akishima, Japan) at 80 kV. The X-ray analysis was done with a JEM2000 FX instrument (JEOL).

FISH. For whole-cell fluorescence in situ hybridization (FISH) of the *Thioploca* from Lake Biwa, the following probes (5′ labeled with fluorescein isothiocyanate [FITC]) were used: EUB338 (for the domain *Bacteria*); ALF19b, BET42a, and GAM42a (for the α, β, and γ subclasses of the class *Proteobacteria*, respectively); SRB385 and SRB385Db (for sulfate-reducing bacteria belonging to the families *Desulfovibrionaceae* and *Desulfobacteriaceae*, respectively, in the δ subclass of the *Proteobacteria*); and 462-Thioploca and 829-Thioploca (for large, marine *Thioploca* spp.). rSRB385, the complement sequence of SRB385, was used as a negative control (2, 3, 22, 28, 34). Fixation, hybridization, and washing procedures were carried out as previously described (2). To compare results obtained with all probes under the exactly same conditions, *Thioploca* samples were set on a Teflon-coated glass slide with 10 windows, and hybridizations with all probes were performed on the slide. Hybridization was carried out at 37°C for 10 h. The concentration of formamide in buffer was adjusted to 20%. The results of FISH with these probes were evaluated by determining fluorescence intensity. Fluorescence images of hybridized *Thioploca* trichomes were obtained with a laser scanning microscope (MRC-600; Bio-Rad, Richmond, Calif.), and fluorescence intensities were measured for 168 trichomes for each probe.

In addition to the previously designed probes, a new probe, Biwa829 (5′-AGGTATACCCTTCCAACGTC-3′), was designed to match specifically the sequence of the Lake Biwa *Thioploca*. This probe targets the same *Escherichia coli* nucleotide positions as the previously designed probe 829-Thioploca for large, marine *Thioploca* spp. (5′-GGATTAATTTCCCCCAACATC-3′), which has 10 mismatches with Biwa829 (34). First, probe Biwa829 was tested with *Beggiatoa alba* DSM 1416 as a negative control in a direct comparison with the Lake Biwa *Thioploca*. Fixed samples of *Thioploca* and *B. alba* were placed on the same slide and hybridized with EUB338, GAM42a, rSRB385, and Biwa829 (also 5′ labeled with FITC). After approximately 4 h of hybridization at 37°C, the slide was washed with washing buffer containing 20% formamide. Following the experiment to confirm specificity and hybridization conditions for Biwa829, another hybridization experiment was performed to compare fluorescence intensities for EUB338, rSRB385, and Biwa829.

DNA extraction, PCR, and denaturing gradient gel electrophoresis (DGGE) of *Thioploca*. To identify the *Thioploca* from Lake Biwa phylogenetically, DNA was extracted from purified trichomes for 16S ribosomal DNA (rDNA) sequencing. *Thioploca* filaments were picked from the sediment, and trichomes were withdrawn from the sheaths with forceps. The trichomes were rinsed in phosphate-buffered saline. The washed trichomes were homogenized in a 1.5-ml Eppendorf tube, with gradual addition of a mixture of 567 μl of Tris-EDTA buffer and 30 μl of 10% sodium dodecyl sulfate. Then 3 μl of a proteinase K solution (20 mg/ml) was added, and this was followed by 60 min of incubation at 37°C. After proteinase K-sodium dodecyl sulfate digestion, 100 μl of 5 M NaCl and 80 μl of a 10% cetyltrimethylammonium bromide–0.7 M NaCl solution were added. After mixing, the sample was incubated for 10 min at 65°C. After centrifugation following addition of 780 μl of chloroform-isoamyl alcohol (24:1, vol/vol) and mixing, the supernatant was transferred to a new tube and mixed with the same volume of phenol-chloroform-isoamyl alcohol (25:24:1, vol/vol/vol). From the aqueous supernatant obtained after centrifugation, DNA was precipitated by addition of isopropanol and centrifugation. The resulting pellets were rinsed with 70% ethanol.

Since it was difficult to completely remove all non-*Thioploca* bacteria before DNA extraction, the 16S rRNA gene of *Thioploca* was selectively amplified with *Thioploca*-specific primers and DGGE primers. Two primer sets were used. Primer ig706F (5′-ATTAGGAGGAACACCAGTGG-3′) was designed based on the sequence of *Thioploca ingrica* and was used in combination with the general bacterial 16S rDNA primer GM4r-GC (5′-GC clamp-TACCTTGTTACGACTT-3′; *E. coli* positions 1492 to 1507) (23). The reverse version of ig706F, ig706R (5′-CCACTGGTGTTCCTCCTAAT-3′), was used in combination with primer GC-109f2 (5′-GC clamp-ACGGGTGAGTAATGYMT-3′), the forward version of primer 109r2 (14). PCR were performed as follows: 5 min of denaturation at 94°C, 1 min of annealing at 65°C, and 3 min of elongation at 72°C. Touchdown cycles were performed by decreasing the temperature in 2-min annealing steps by 1°C every second cycle, from 65 to 55°C. DGGE was performed as previously described (24). The products of PCR obtained with each primer set were analyzed by DGGE to confirm that only a single fragment was obtained; then the sequences were determined. Since these two primer sets amplified two mutually nonoverlapping portions of the 16S rRNA genes, it was necessary to confirm that the partial sequences were derived from the same 16S rRNA gene. The overlap region was amplified with newly designed primers GC-Biwa160F (5′-GC clamp-ATAAGTCTTTTTTAACGAAA-3′) and Biwa829R (5′-AGGTATACCCTTCCAACGTC-3′). The resulting PCR product was also analyzed by DGGE and then sequenced.

For phylogenetic identification of the *Thioploca* from Lake Constance, DNA was extracted from washed filaments (not from sheath-free trichomes), and 16S rRNA gene fragments were amplified with three primer sets as described above. Since it was not known whether these primers matched the sequence of the *Thioploca* from Lake Constance, the stringency of the PCR conditions was lowered. Amplification was performed for 30 cycles, with each cycle consisting of 2 min of denaturation at 94°C, 1.5 min of annealing at 45°C, and 2 min of elongation at 72°C. The PCR fragments were checked by DGGE and sequenced.

Phylogenetic analysis. The 16S rRNA sequences of the thioplocas from Lake Biwa and Lake Constance were aligned with other γ- and ε-proteobacterial 16S rRNA sequences by using the sequence editor SeqPup (8). Positions 135 to 1487 of the 16S rRNA gene (*E. coli* numbering) were used for phylogenetic analysis, and an analysis with the partial region from position 358 to position 906 was also performed to include marine *Thioploca* species and *Thiomargarita*. PCR primer positions were excluded. Phylogenetic trees were inferred with the program PAUP 4.0* (33). The minimum evolutionary tree for the Lake Biwa *Thioploca* and the Lake Constance *Thioploca* was obtained by using the Kimura two-parameter model and was checked with 1,000 bootstrap replicates in minimum evolution and parsimony analyses. Members of the ε subclass of the *Proteobacteria* were used as outgroups; these organisms included two clones obtained from a filamentous bacterial mat at the 17 Southern East Pacific Rise hydrothermal vents and the filamentous episymbiont of the vent invertebrates *Rimicaris exoculata* and *Alvinella pompeiana*.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of the Lake Biwa *Thioploca* sp. and the Lake Constance *Thioploca* sp. have been deposited in the GenBank database under accession no. AF452892 and AY115530, respectively.

RESULTS

Morphological characteristics. *Thioploca* filaments were easily picked from the surfaces of sediment samples with a pair of forceps. Individual *Thioploca* sheaths harbored from 1 to 75 trichomes. Numerous sulfur inclusions were observed in trichomes (Fig. 1). The trichome tips were tapered. The trichomes of the *Thioploca* from Lake Biwa had a mean diameter of 3.93 μm (standard deviation, 0.555 μm; range, 3.0 to 5.6 μm; $n = 102$). The sheath diameters ranged from 18 to 75 μm, and the mean diameter was 49.5 μm (standard deviation, 14.26 μm; $n = 35$). The cell lengths ranged from 1.2 to 3.8 μm, and the mean cell length was 2.91 μm (standard deviation, 0.635 μm; $n = 16$). Active gliding movement of trichomes was observed. Some trichomes occasionally protruded from the open end of a sheath. Each trichome glided independently; simultaneous gliding movements with different directions and speeds were observed for trichomes sharing the same sheath. The mean gliding rate was 3.0 $\mu m \cdot s^{-1}$ at 10°C. The sheaths showed constricted zones and sometimes were bent at these constricted zones, as described previously for *T. ingrica* (37). The intervals between constrictions seemed to be random. Based on these characteristics, the *Thioploca* from Lake Biwa resembled most closely the species *T. ingrica*. The morphology of the *Thioploca* from Lake Constance was similar to that of the *Thioploca* from Lake Biwa. The trichomes had a mean

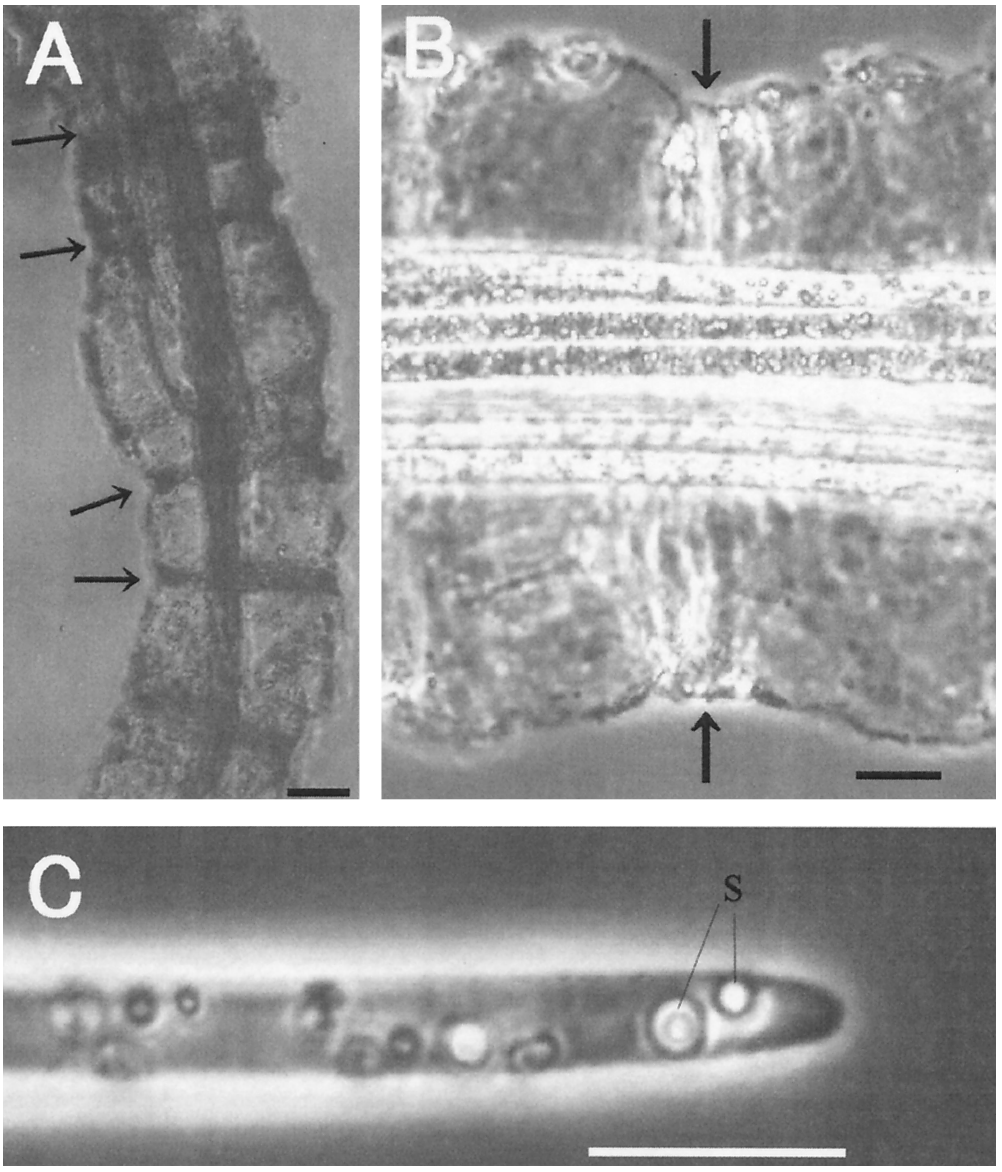

FIG. 1. Micrographs of the *Thioploca* from Lake Biwa. (A) Sheath having a relatively small diameter. The arrows indicate constrictions. (B) Phase-contrast image of a wide sheath with trichomes. A constriction is indicated by arrows. (C) Phase-contrast image of a trichome tip. S, sulfur droplets. Bars = 10 μm.

diameter 4.2 μm, as determined with 58 individual trichomes that ranged from 3.0 to 5.2 μm in diameter.

TEM and X-ray analysis. TEM micrographs of the *Thioploca* from Lake Biwa are shown in Fig. 2. Numerous *Thioploca* trichomes (in this example approximately 60 trichomes) formed a single bundle (Fig. 2A). The sheath surrounding the trichomes had a striated, fibrous texture (Fig. 2B), similar to the texture of the sheath material of *T. ingrica*, as described by Maier and Murray (21). In addition to sulfur globules, the cells contained high numbers of dense round particles that were 100 to 200 nm in diameter, which were stained intensively with uranyl acetate (Fig. 2C and D). The TEM specimens were also examined by X-ray analysis, but the unidentified dense particles did not show any positive signal for phosphorus or sulfur. Similar globules that were 100 to 200 nm in diameter (and not stained with lead tartrate) were also abundant in the cytoplasm of *T. ingrica* cells (21). These particles may be polyhydroxybutyrate globules or another storage compound that is free of sulfur and phosphorus. In microscopic and ultrastructural studies of *Beggiatoa* strains, the cytoplasm frequently contained conspicuous polyhydroxybutyrate globules (16).

Phylogenetic affiliation. For the sample of the *Thioploca* from Lake Biwa, the products of PCR performed with two primer sets (GC-109f2 and ig706R; ig706F and GM4r-GC) were analyzed by DGGE, and they produced only a single band each. After sequencing of both DGGE fragments, which corresponded to two nonoverlapping halves of the 16S rRNA gene, new primers were designed for PCR amplification of an overlapping sequence region (Biwa160F and Biwa829R). Amplification of the overlapping region with the new primer set

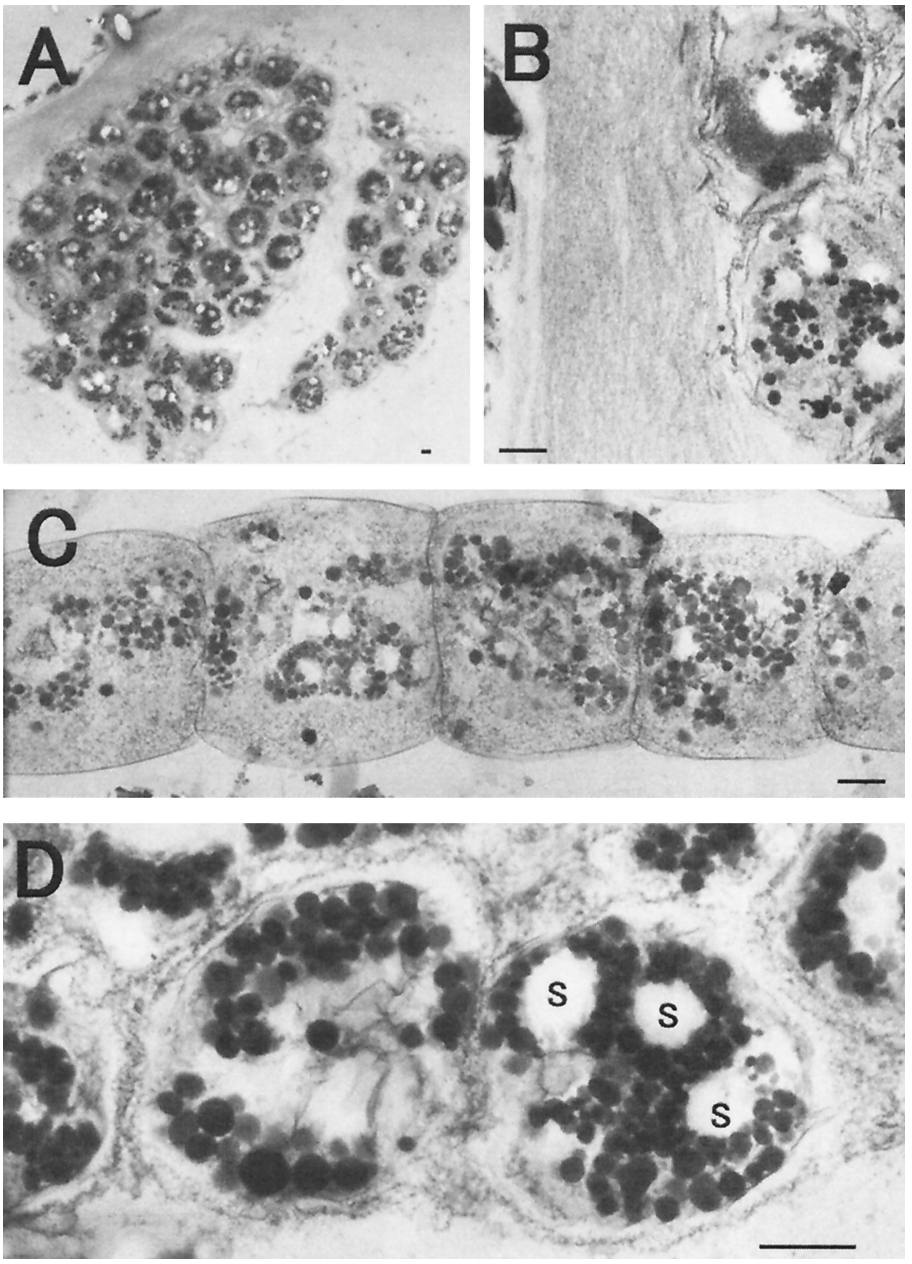

FIG. 2. TEM micrographs of the *Thioploca* from Lake Biwa. (A) Cross section of *Thioploca* filament containing multiple trichomes. (B) Material between sheath and trichomes. (C) Longitudinal section of trichome. (D) Longitudinal cross section of a *Thioploca* trichome, with individual cells separated by cell walls. White sulfur droplets (S) were present in the cells. In panels B to D intracellular dense particles stained with uranyl acetate appear as black particles. Bars = 500 nm.

resulted in a single DGGE band, which matched the sequences of the two partial 16S rRNA gene sequences. Based on the consensus, the three sequences were combined to obtain a nearly complete 16S rDNA sequence for the Lake Biwa *Thioploca*.

From the Lake Constance sample, fragments of 16S rDNA were amplified with three primer sets (GC-109f2 and ig706R; GC-Biwa160F and Biwa829R; ig706F and GM4r-GC). The products obtained with two of the primer sets gave only one DGGE band each, but the products obtained with primers ig706F and GM4r-GC gave two DGGE bands. These four bands were sequenced, and three of them matched in the overlapping sequence regions and were combined to obtain a nearly complete 16S rDNA sequence. There was one mismatch between Biwa160F and the sequence of the corresponding position determined with GC-109f2 and ig706R, but PCR performed at a lower stringency allowed positive amplification with primers GC-Biwa160F and Biwa829R. The additional DGGE band obtained with primers ig706F and GM4r-GC was closely related to *Corynebacterium tuberculostearicum* (accession no. X84247), a gram-positive bacterium that had three mismatches with ig706F. This band represented bacterial con-

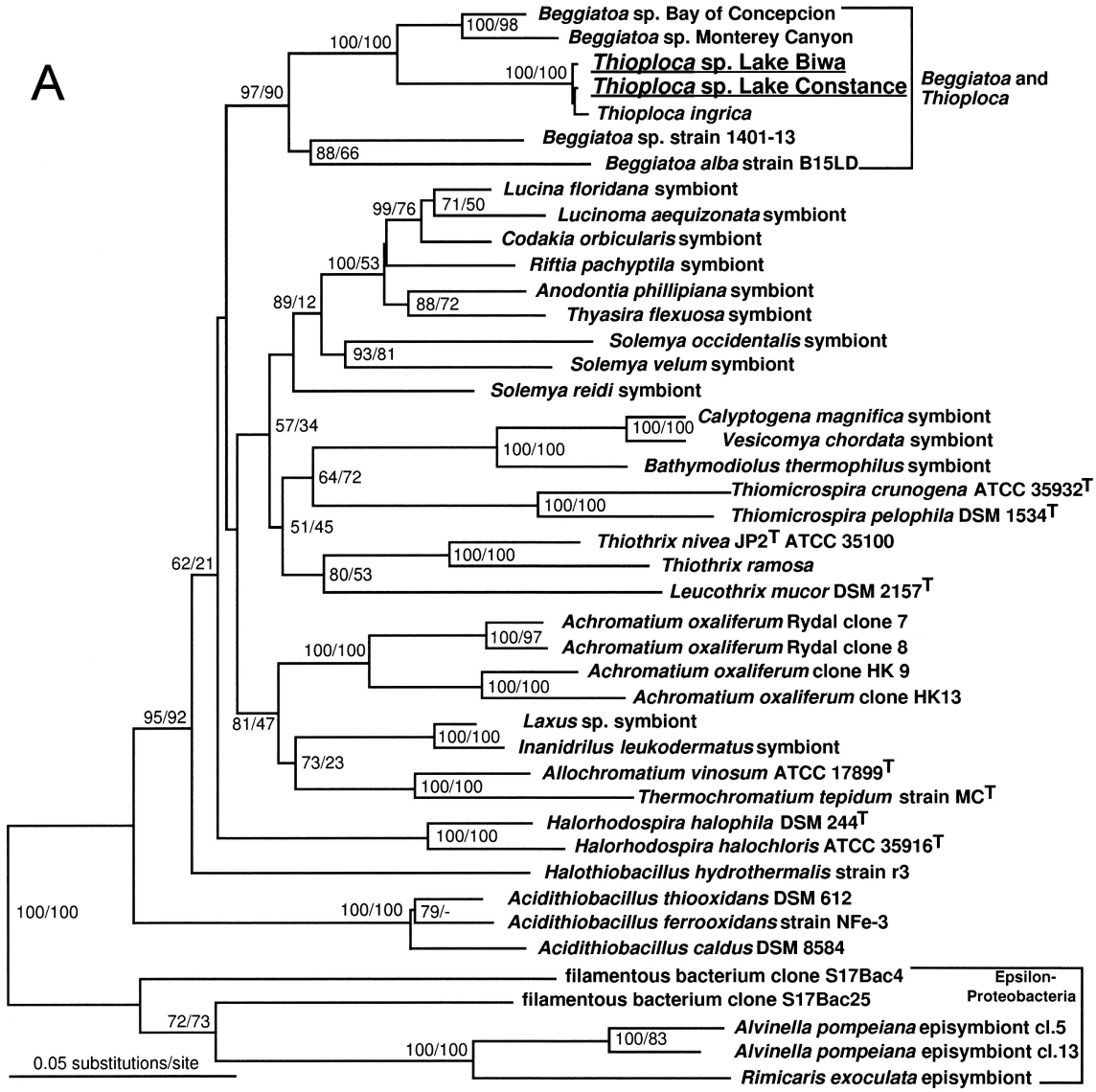

FIG. 3. Phylogenetic relationships of the *Thioploca* spp. from Lake Biwa and Lake Constance based on nearly complete 16S rDNA sequences (*E. coli* positions 135 to 1487) (A) and on partial sequences (*E. coli* positions 358 to 906), including those of marine *Thioploca* species and *T. namibiensis*, for which only partial sequences are available (B). Bootstrap values (minimum evolution/parsimony) are indicated at the nodes with more than 50% bootstrap support.

taminants, most likely in the sheath material of the complete *Thioploca* filaments (sheath plus trichomes) that were used to extract DNA from the Lake Constance samples.

The phylogenetic analysis based on nearly complete sequences showed that the thioplocas from Lake Biwa and Lake Constance are closely related to *T. ingrica* (Fig. 3A). These organisms form a branch separate from the large, marine, vacuolated, nitrate-accumulating *Beggiatoa* and *Thioploca* spp., which are represented by the *Beggiatoa* spp. from the Bay of Concepcion and from Monterey Canyon (1, 35). The same result was obtained by analysis of partial 16S rDNA sequences, including those of the large, vacuolated, nitrate-accumulating sulfide oxidizers *Thioploca araucae*, *Thioploca chileae* (34), and *Thiomargarita namibiensis* (30), which group together with large, marine, nitrate-accumulating *Beggiatoa* species (Fig. 3B).

FISH analysis of *Thioploca* sp. The 16S rDNA sequence analysis results were supported by the FISH results. The results of FISH with previously designed probes are summarized in Table 1. Hybridizations with probes that do not match the rRNA sequence of the Lake Biwa *Thioploca* sp., including the nonsense probe rSRB385, did not give hybridization signals that were greater than a common level of autofluorescence (obtained with probes ALF19b, BET42a, SRB385, SRB385Db, and rSRB385). Hybridizations with probe EUB338 and the γ-proteobacterial probe GAM42a resulted in strongly positive fluorescence hybridization signals. Probe 829-Thioploca, previously designed for large marine *Thioploca* species, gave a negative result, which is consistent with numerous mismatches with the *Thioploca* from Lake Biwa (10 mismatches in 21 bases). The relatively high intensity obtained with probe 462-

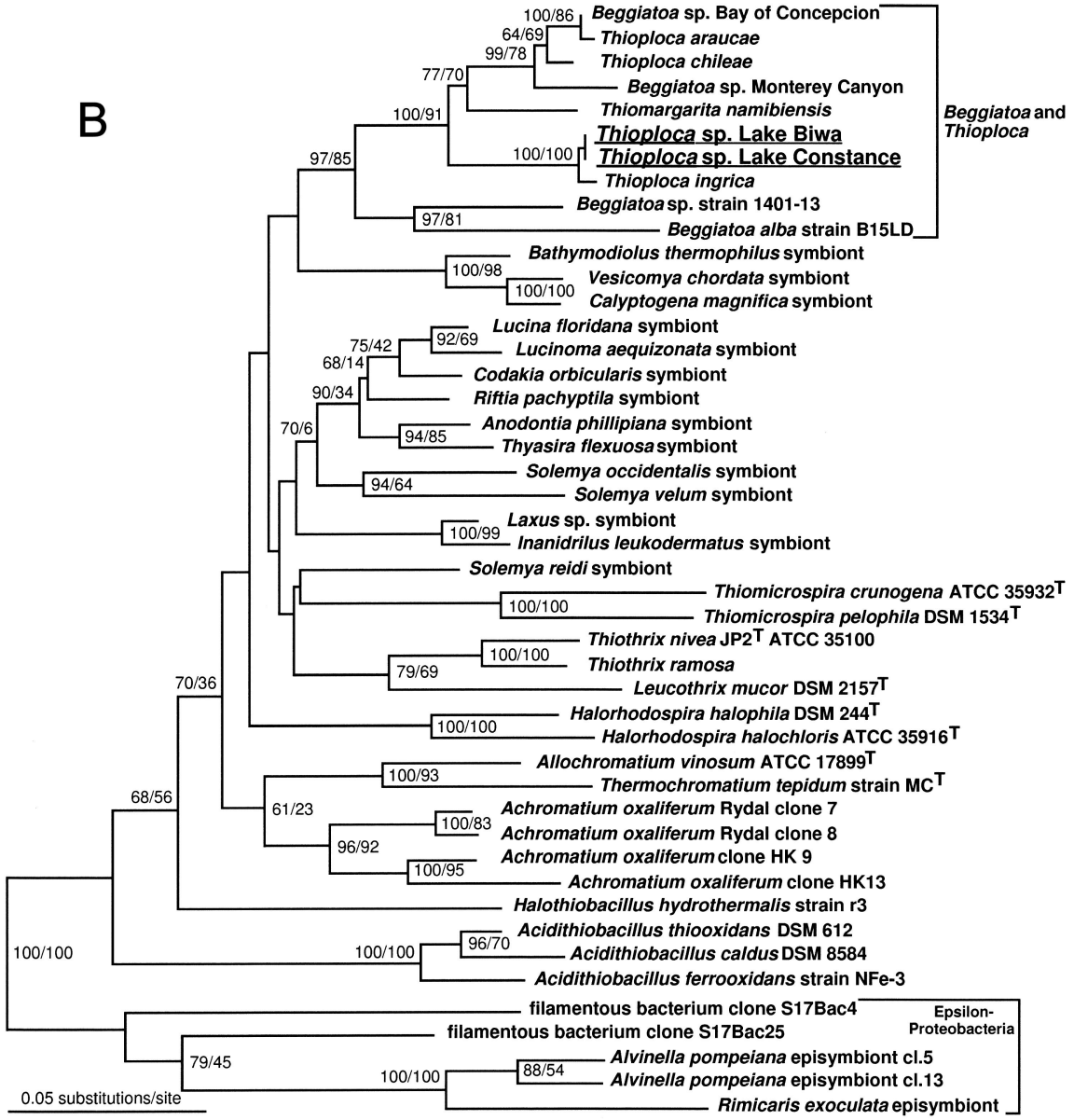

FIG. 3—*Continued.*

Thioploca was unexpected, since this probe has six mismatches in 23 bases. This finding may be related to the fact that the hybridizations were performed with 20% formamide in order to compare the results with the results obtained with other probes under the same conditions. However, the original tests of probe 462-Thioploca with marine *Thioploca* spp. showed that there was detectable hybridization with 20% formamide (34), which was therefore chosen as the negative control hybridization stringency for freshwater *Thioploca* spp. Further testing of probe 462-Thioploca is needed in order to evaluate its reliability for *Thioploca* hybridization.

In the experiment with the newly designed probe, the *B. alba* reference strain gave positive hybridization results with EUB338 and GAM42a and negative results with probes rSRB385 and Biwa829. Under exactly the same conditions on the same slide, *Thioploca* specimens gave much higher hybridization intensities with probes EUB338, GAM42a, and Biwa829 than with the negative control probe rSRB385.

Under the verified hybridization conditions, fluorescence intensities were measured for EUB338, Biwa829, and rSRB385 (86 to 106 trichomes for each probe). This experiment was performed with samples that had been stored several months, and the level of autofluorescence seemed to be decreased by storage; the difference between the intensities observed with EUB338 and rSRB385 was larger than the difference in the experiment performed with fresh samples. The mean fluorescence intensity observed with Biwa829 was quite similar to that observed with EUB338, and the intensities were significantly greater than the intensity observed with rSRB385 (Table 1 and Fig. 4). Therefore, newly designed probe Biwa829 that

TABLE 1. Fluorescence intensities of FISH results

Probe	Pixel intensity		*n*	Significance of difference[a]
	Mean	SD		
Expt with fresh samples				
EUB338	172	17.7	168	+
ALF19b	121	23.5	168	
BET42a	112	17.8	168	
GAM42a	165	26.8	168	+
SRB385	106	18.5	168	
SRB385Db	107	19.1	168	
462-Thioploca	145	23.1	168	+
829-Thioploca	119	15.0	168	
rSRB385	106	20.7	168	
Expt with stored samples				
Biwa829	163	59.1	86	+
EUB338	161	54.5	106	+
rSRB385	38	8.12	103	

[a] Significance levels were calculated for the probes giving intensities greater than those obtained with ALF19b in the Experiment with fresh samples and greater than those obtained with rSRB385 in the Experiment with stored samples (the probes which provided the highest background levels in the two experiments) by using the Cochran-Cox method. The P value used was <0.001. +, significantly different from ALF19b (in the experiments with fresh samples) or rSRB385 (in the experiments with stored samples).

matched the Lake Biwa *Thioploca* 16S rRNA sequence gave a positive signal and indicated that the sequence obtained by PCR from the Lake Biwa *Thioploca* was indeed the correct 16S rRNA sequence of this organism.

DISCUSSION

The classification of species in the genus *Thioploca* has been based on trichome diameter and habitat preference. Three types of *Thioploca* were found in Lake Constance in the early 20th century, *Thioploca schmidlei* (trichome diameter, 5 to 9 μm), *T. ingrica* (trichome diameter, 2 to 4.5 μm), and "*Thioploca minima*" (trichome diameter, 0.8 to 1.5 μm) (13, 15); in a later study the researchers found only *T. ingrica* in Lake Constance (18). Based on trichome diameter (3.9 and 4.2 μm), the *Thioploca* sp. from Lake Biwa and the *Thioploca* sp. from Lake Constance that were examined in this study are most similar to *T. ingrica*. Besides the similarity in trichome diameter, the thioplocas examined in this study share another morphological feature with *T. ingrica*, sheath constrictions, which have never been reported in marine species. These morphological similarities are consistent with 16S rDNA sequence data. The sequenced specimen of *T. ingrica* (from Randersfjord, Denmark) and the Lake Biwa and Lake Constance *Thioploca* spp. are closely related, with very few 16S rRNA mismatches. These three thioplocas form a tight phylogenetic cluster (100% bootstrap support) apart from the lineage of the large, marine *Thioploca* and *Beggiatoa* species (>70% bootstrap support) (Fig. 3). So far, these two phylogenetic groups seem to be consistent in terms of morphology and habitats. However, a *Thioploca* sp. with a relatively large trichome diameter has been found in Lake Baikal, a Russian freshwater lake (38), and a marine *Thioploca* sp. with narrow trichomes has been reported from the coast of Chile (31). The 16S rRNA sequences and phylogenetic positions of these interesting morphotypes are not known yet.

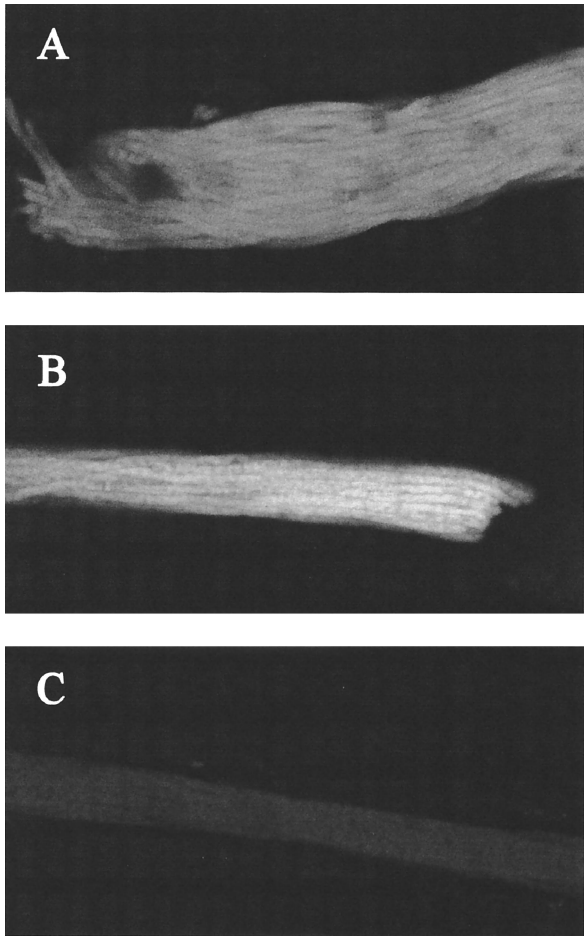

FIG. 4. FISH hybridization images for the *Thioploca* from Lake Biwa, obtained with probes labeled with FITC. (A) Bundle of trichomes labeled with probe EuB338. (B) Bundle of trichomes labeled with Biwa829. (C) Negative control with rSRB385. All photographs were taken under the same conditions. Positive fluorescence signals were obtained with probes EuB338 and Biwa829 (A and B). In the negative control, trichomes showed a small amount of autofluorescence independent of the excitation wavelength and filter (C).

Analyses of 16S rRNA sequences for the *Thioploca* community in the sediment off the coast of Chile have shown that several distinct morphotypes of *Thioploca* are also genetically distinct (32, 34). On the other hand, a large Chilean *Beggiatoa* sp. and *T. araucae*, which have similar trichome diameters, have almost identical 16S rRNA sequences (35). These data suggest that there is a correlation between morphological similarity, based primarily on trichome diameter and cell dimensions, and 16S rRNA relatedness. The data for the Lake Biwa and Lake Constance *Thioploca* spp. support this working hypothesis. These two *Thioploca* spp. from two widely separated locations, Lake Biwa in Japan and the German part of Lake Constance, are hardly distinguishable by mean trichome diameter (3.9 versus 4.2 μm) and morphology and are closely related on the basis of their 16S rRNA sequences (two mismatches). Similar cosmopolitan occurrence patterns for other sulfur bacteria are not unprecedented. Very closely related hydro-

thermal vent strains of the genus *Thiomicrospira* were found at the Mid-Atlantic Ridge and at the East Pacific Rise (36).

In Lake Biwa and Lake Constance, all three 16S rDNA-targeted DGGE-PCR primer combinations yielded a single *Thioploca* DGGE band, indicating that the *Thioploca* populations are genetically homogeneous at the sampling sites in Lake Biwa and Lake Constance. In contrast, *Thioploca* spp. of several types coexist sympatrically in the Chilean *Thioploca* mats (31, 32). Also, sulfur-oxidizing *Achromatium* populations in a single freshwater lake showed considerable genetic diversity at the level of 16S rDNA (9). In principle, the possibility of different genotypes of *Thioploca* in Lake Biwa and Lake Constance cannot be eliminated, and the genetic diversity of *Thioploca* in freshwater habitats should be the subject of further studies. For example, it would be interesting to locate a population of the freshwater type species, *T. schmidlei*, with a larger trichome diameter and, if the morphotype-genotype correlation holds, a divergent 16S rRNA sequence.

A sufficient supply of hydrogen sulfide is needed for sustaining *Beggiatoa* or *Thioploca* mats. In some habitats, such as hydrothermal vents, sulfide of geothermal origin is provided (25). In benthic sediments off the coast of Chile and other localities, sulfide is produced by high rates of sulfate reduction, based on the oxidation of abundant organic matter by sulfate-reducing bacteria in sulfate-rich seawater sediments (4). However, *Thioploca* spp. can be found in relatively sulfate-poor, freshwater lakes. For continuous existence of these organisms, highly efficient sulfide uptake mechanisms could be involved, similar to those in Chilean marine *Thioploca* spp. which take up sulfide at very low external sulfide concentrations (10), or the organisms could receive sulfide from sulfate-reducing *Thioploca* epibionts (6). The freshwater *Thioploca* of Lake Biwa provides an opportunity to compare marine and freshwater *Thioploca* species and to understand habitat-related differences in the physiology and ecology of these organisms.

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